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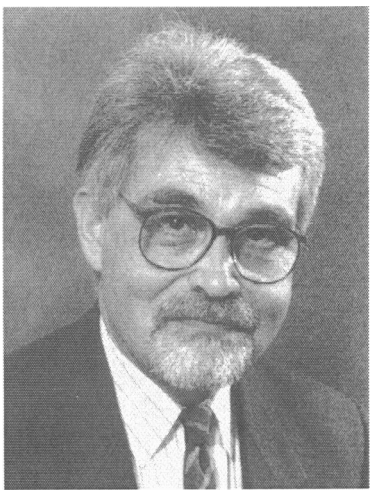

Professor K. M. Spyer

Annual Review Prize Lecture

Central nervous mechanisms contributing to cardiovascular control

Given at the Meeting of the Physiological Society held at University College London on 1 July 1993

K. M. Spyer*

In 1963 when I entered physiology as an earnest undergraduate there was a belief that certain areas of physiology were at a stage where current knowledge, if not perfect, was at least sufficient to provide a working basis on which to describe homeostatic mechanisms. Cardiovascular control certainly fell into such a category, and yet the last thirty years have witnessed a major review of most of the basic experimental 'facts' that were considered to underlie these ideas. These more recent investigations are generating more questions than providing answers. It is indeed strange that the Introduction to a previous Review Lecture, entitled *All Hands to the Sodium Pump*, by Ian Glynn (1993), should in its first page contain a quotation from Overton (1902) relating to the contraction of the heart and respiratory muscles that indicates the dimensions of the problem faced by those trying to understand how cardiorespiratory homeostasis is achieved in the life of an individual – 'Consider that in the course of 70 years, heart muscles contract about 24×10^8 times and the respiratory muscles about 6×10^8 times.' How these two physiological actions are integrated to allow the individual to accomplish major behavioural changes with their associated changes in metabolic demand is still unresolved. Changes in cardiac output are produced usually without equivalently large changes in arterial pressure but are invariably paralleled by appropriate changes in respiratory minute volume.

Three distinguished members of the Physiological Society with different interests, and personalities, were major figures in stimulating the advances that have taken place in approaches to the role of the CNS in the control of the cardiovascular system. First, Eric Neil elaborated the role of cardiovascular and respiratory afferent inputs in modifying cardiovascular activity and drew attention to the essential interplay between cardiovascular and respiratory control mechanisms. This was wonderfully reviewed in the now classic monograph *Reflexogenic Areas of the Cardiovascular System* that he produced with Cornelius Heymans (Heymans & Neil, 1958). While never really entering the CNS experimentally, Neil fully appreciated the potential of integrative actions between the cardiovascular and respiratory control systems, probably as a consequence of his prodigious knowledge of the classic 19th century and early 20th century literature.

The second major influence was W. Feldberg. He always professed to be unaware of the literature, but as he always spoke of the 'vasomotor' centre being near to the dorsal surface of the medulla, he must have had at least a rudimentary appreciation of the work of Alexander (1946), who appeared to have uncovered the substrate for the 'vasomotor' centre first outlined by Bayliss (1923) largley on the basis of the studies of Ludwig and his school (Fig. 1*C*). As a result of a study by Guerzenstein & Silver (1974), Feldberg was attracted to investigate, initially with Guerzenstein, the pharmacological sensitivity of areas of the ventral surface of the medulla oblongata close to, or overlapping with, those that Loeschcke (1982) had studied extensively in the context of central chemosensitivity. Feldberg summarized these studies in his Sherrington Lectures (Feldberg, 1982). He showed that the bilateral application of pentobarbitone sodium or glycine onto a discrete region of the ventral surface of the medulla just caudal to the trapezoid body induced a hypotensive response (see Fig. 1*A* and *B*). Blood pressure fell to the level of the spinal animal – an effect which was similar to that observed by Alexander on transecting the medulla above the first cervical segment, which removed the connections between the medulla and sympathetic preganglionic neurones of the spinal cord. This suggested to Feldberg, and others (see Guyenet, 1990, for a review), that they had in fact identified the 'vasomotor' centre as a discrete set of neurones on the ventral aspect of the medulla. In other studies, neurones in this region had been shown, using the retrograde transport of horseradish peroxidase, to project to the intermediolateral cell column of the spinal cord (Amendt, Czachurski, Dembowsky & Seller, 1979). Accordingly the knot appeared to have been tied, but as we will see nothing is ever that simple in science.

The third, and most direct, personal influence was Sydney Hilton, who together with A. Zbrozyna during his period at the National Institute for Medical Research, Mill Hill, and subsequently in Birmingham, had shown that the defence reaction first delineated by Cannon (1932) and Hess & Brugger (1943) involved a patterned cardiovascular response

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that was organized within the hypothalamus and amygdala. This led to the notions that cardiovascular control was organized in a longitudinal arrangement throughout the brainstem (Hilton, 1975), that ultimate regulation of the sympathetic and vagal outflows involved the operation of descending and, as we will see, often parallel pathways, and that these do not necessarily involve the 'vasomotor' centre. In this regard C. N. Peiss in Chicago had provided an equally powerful experimental, physiological and anatomical, and intellectual, evocation of such an architecture of cardiovascular control (Peiss, 1965). Hilton's contribution was to recognize the importance of the CNS in establishing patterns of cardiovascular response that were appropriate for the behavioural context of the individual and, further, to infer that during such responses homeostatic reflexes might be modified, or indeed abrogated (Hilton, 1966). In this regard there was a level of concurrence between the ideas of Hilton and Neil, and this has proved a fruitful area of investigation that has been exploited by my laboratory over the succeeding years. These three strands of investigation and the conceptual framework in which they were conceived – the reflex, the 'centre' and the integrative – have dominated the studies of the last thirty years in numerous laboratories. This review will endeavour to present a balanced survey of our current understanding of the operation of cardiovascular control but will naturally be dominated by this author's experimental interests, which have been fuelled by experimental approaches that have grown out of this rich background.

The vasomotor centre revisited

The belief that cardiovascular control, and specifically 'vasomotor' tone, was dependent on the activity of neurones located within the lower brainstem has a long history. It was recognized firstly on the basis of transection and lesion experiments (reviewed by Spyer, 1981*a*) and subsequently through the results of the study by Alexander (1946) alluded to before. It was proposed that two half-centres located within the medulla were connected reciprocally (Fig. 1*C*). These two half-centres, pressor and depressor, were considered to be subject to control from reflex afferent inputs and descending inputs from higher centres. The net result of their interactions was to modify a descending excitatory input to the spinally located sympathetic preganglionic neurones that innervate the postganglionic neurones which eventually provide input to either cardiac or vascular muscles. Essentially 'vasomotor' tone, and presumably sympathetic drive to

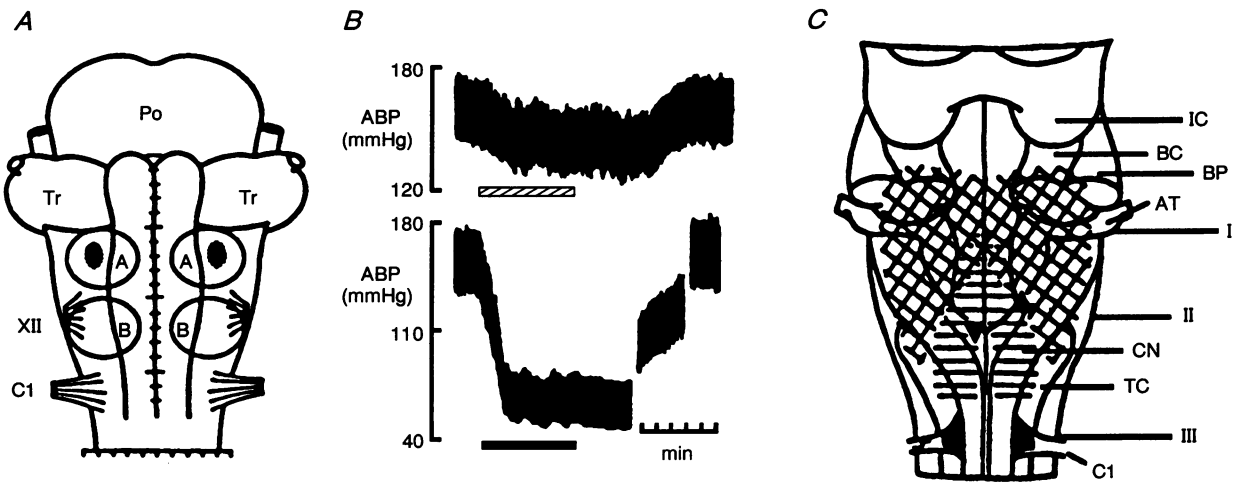

Figure 1.
A, diagram of the ventral surface of the cat's brainstem. The ovals indicate the bilateral areas covered by paired Perspex rings in two positions. Position A gives the glycine-sensitive but nicotine-insensitive area, the glycine-sensitive area actually being restricted to the filled area. Position B gives the nicotine-sensitive but glycine-insensitive area. The vertical and horizontal scales are in millimetres. C1, first cervical nerve; Po, pons; Tr, trapezoid body. (Reproduced and modified from Feldberg & Guertzenstein, 1976.) *B*, arterial blood pressure (ABP; in mmHg) obtained from a cat anaesthetized with pentobarbitone sodium and artificially ventilated. The horizontal bars indicate periods of unilateral (hatched bar) and bilateral (filled bar) application of a 0·1% solution of pentobarbitone sodium to the ventral surface of the cat's brain. (Reproduced and modified from Feldberg & Guertzenstein, 1972.) *C*, localization of pressor and depressor centres in the brainstem of the cat. Pressor regions indicated by cross-hatching, depressor regions by horizontal hatching. Semi-diagrammatic projection of pressor and depressor regions onto the dorsal surface of the brainstem viewed with the cerebellar peduncles cut across and the cerebellum removed. Abbreviations: AT, auditory tubercles; BC, brachium conjunctiva; BP, brachium ponti; CN, cuneate nucleus; IC, inferior colliculus; TC, tuberculum cinereum; I, II, III, levels of transection discussed in text. (Reproduced and modified with permission from Alexander, 1946.)

the heart, was dictated by the changing level of activity of this descending excitatory pathway. Thus the baroreceptor control of sympathetic discharge was considered to be mediated by disfacilitation since sympathetic neurones might have some intrinsic activity or be driven by spinally generated excitatory inputs. To achieve an overall control of the cardiovascular system it is only necessary to include the vagal preganglionic supply to the heart which, as will be revealed, is localized within the pressor region of the 'vasomotor' centre in the anatomical model proposed by Alexander (1946). Critical re-evaluations of this model are numerous but the discovery that there is a distinct group of bulbospinal neurones in the rostroventrolateral medulla (RVLM) in cat and rat (reviewed by, amongst others, Guyenet, 1990; Gebber, 1990; Dampney, 1994) allied to Feldberg's observations led to a sudden reacceptance by

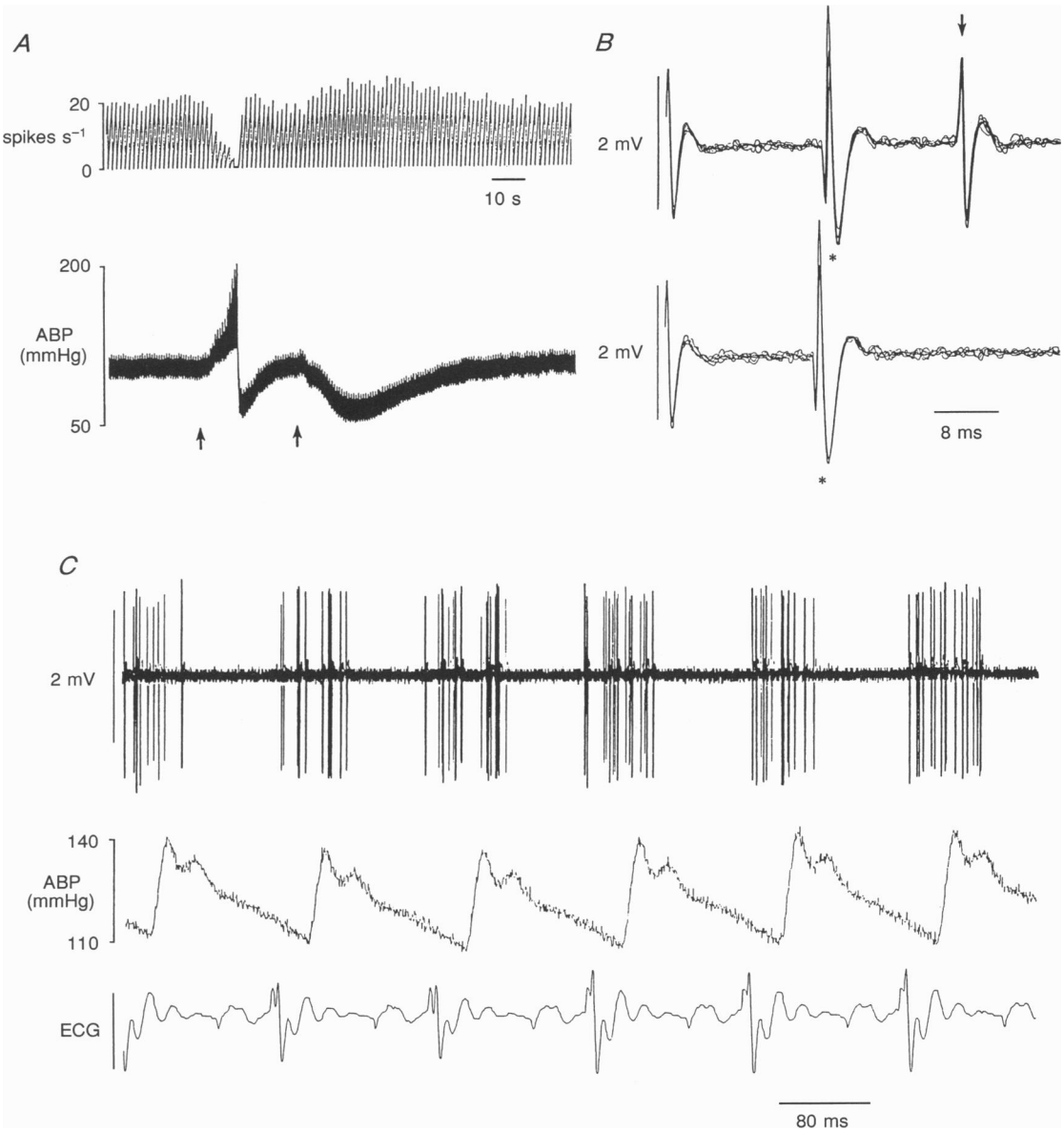

Figure 2. Characteristics of medullary vasomotor neurones
A, effect of change in arterial pressure on unit activity. Neuronal activity is represented in the form of an integrated rate histogram. Arterial blood pressure (ABP) was elevated by constriction of the descending aorta (at first arrow) and lowered by an I.V. injection of sodium nitroprusside at the second arrow. *B*, collision test used to identify medullary vasomotor units as reticulospinal neurones. Traces were triggered from spontaneously occurring spikes. Antidromic spikes (arrow in top trace) from the spinal cord failed to occur (lower trace) when stimulation was delivered within the critical period after trigger spikes (six superimposed traces each). *C*, pulse-synchronous discharge of the medullary vasomotor neurone. The record was triggered from the R wave of the ECG signals (lower trace). Top trace is neuronal discharge and the middle trace is arterial pressure. (Reproduced from Sun & Spyer, 1991.)

many of the existence of an all-embracing 'vasomotor' centre.

Electrophysiological studies have confirmed the existence of bulbospinal neurones within the RVLM that have on-going activity which is related closely to the level of arterial blood pressure (see Fig. 2). These neurones were identified antidromically on electrical stimulation within the intermediolateral cell column of the thoracic spinal cord (IML), which contains the preganglionic sympathetic neurones (Barman & Gebber, 1985; McAllen, 1985; Brown & Guyenet, 1985; and Guyenet 1990, for review). These neurones, which have small myelinated or unmyelinated axons, have been shown to be inhibited powerfully by baroreceptor activation, to be excited by chemoreceptor afferent inputs and to receive excitatory or inhibitory inputs from other afferent and central sites that modify cardiovascular activity. Indeed the level of their on-going discharge is dependent particularly on baroreceptor input and in consequence these neurones fire with a cardiac rhythm (Guyenet, 1990). Furthermore, many RVLM bulbospinal neurones exhibit 'pacemaker-like' activity (Guyenet, 1990; Granata & Kitai, 1992).

Much excitement was aroused by the fact that a proportion of these neurones was shown immunocytochemically to contain adrenaline (the C1 group), and this has fuelled a controversy as to whether these adrenergic neurones contribute to the control of sympathetic activity or whether the bulbospinal innervation of the IML utilizes glutamate as its transmitter (Ross, Ruggiero, Joh, Park & Reis, 1983). Granata & Kitai (1992) have provided strong indications from an *in vivo* intracellular recording study in the rat that it is non-adrenergic neurones that are barosensitive. The pharmacology of sympathetic preganglionic neurones is being actively investigated (reviewed by Coote, 1988). Recent experiments in our laboratory (Marks & Morrison, 1993), using a novel preparation of the neonatal rat – an *in vitro* brainstem–spinal cord preparation – have shown that monosynaptic EPSPs can be evoked in sympathetic preganglionic neurones on stimulating in the RVLM, and that these are mediated by an excitatory amino acid. This input has two components, a fast component mediated by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione)-sensitive receptors (non-NMDA) and a slower component antagonized by AP-5 (2-amino-5-phosphonopentanoic acid) and hence involving NMDA receptors. While other *in vitro* studies have shown potent actions of catecholamines on the activity of sympathetic preganglionic neurones, the physiological role of this innervation is unresolved (reviewed in Coote, 1988; see also Marks & Gilbey, 1992).

McAllen (1985, 1986*a*, *b*, *c*, 1987) has made some extremely important contributions to this field, first by showing that the RVLM neurones often had respiratory-related rhythms in their discharge, a finding subsequently confirmed by others (Guyenet, 1990). This has considerable significance for the role of the CNS in cardiorespiratory homeostasis, as will be revealed later. Further, he provided tentative indications that groups of neurones within the RVLM were designated for vascular territory, implying a viscerotopic map within the medulla (McAllen & Dampney, 1989, 1990). This remains a controversial assertion and awaits a detailed neurophysiological confirmation. These observations together

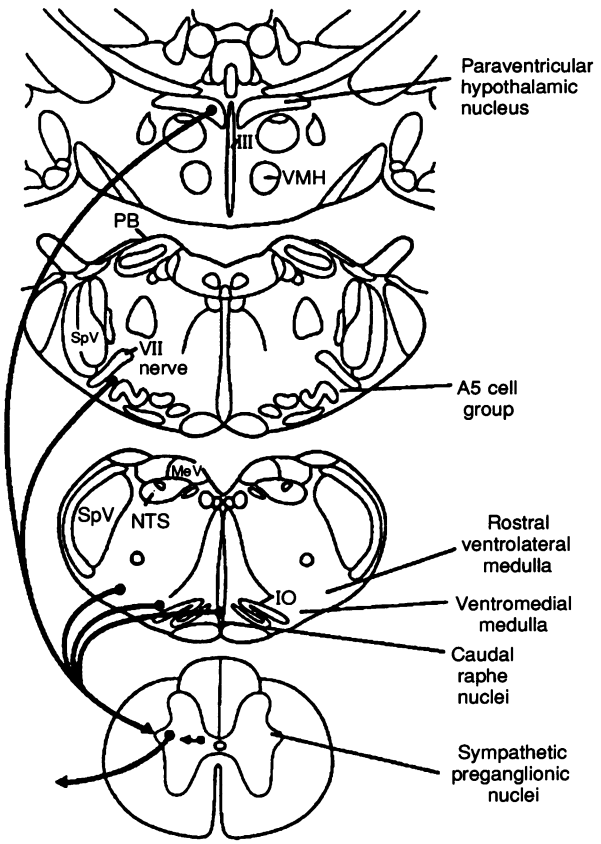

Figure 3. The general pattern of innervation of the sympathetic outflow as demonstrated by the retrograde transneuronal viral cell body labelling technique
Abbreviations: III, third ventricle; IO, inferior olive; MeV, medial vestibular nucleus; NTS, nucleus tractus solitarii; PB, parabrachial nucleus; SpV, spinal trigeminal nucleus; VMH, ventromedial hypothalamic nucleus. (Reproduced with permission from Strack, Sawyer, Hughes, Platt & Loewy, 1989*a*.)

could be taken to imply that physiologists and anatomists were as one in establishing the fundamental organization of CNS sympathetic control. In fact such a simplistic view is clearly incorrect since lesion studies in the rat have shown that only transient changes in both blood pressure and sympathetic activity accompany destruction of the RVLM (Cochrane & Nathan, 1993). Further studies have revealed that the IML is richly innervated by axons descending from numerous brainstem nuclei in addition to the RVLM (see Fig. 3). This pattern of innervation appears to be similar, if not the same, for sympathetic preganglionic neurones concerned with the control of several end-organs, on the basis of evidence accumulated from studies using the transneural transport of pseudorabies virus from injection sites in peripheral tissues (Strack *et al.* 1989*a*; Strack, Sawyer, Platt & Loewy, 1989*b*). In particular, the detailed morphological studies of Smith's group in Oxford have provided an ultrastructural and chemical basis for the raphe innervation of the IML (Bacon, Zagon & Smith, 1990) and input from the RVLM (Zagon & Smith, 1993), and indications of descending input from the medial frontal cortex to the central autonomic area of the spinal cord that contacts the IML (Bacon & Smith, 1993).

This anatomical framework, implying the existence of multiple descending pathways, is supported by electrophysiological evidence that neurones located within the regions of the CNS from which these pathways originate have firing patterns that are closely correlated with the pattern of discharge of sympathetic efferent neurones. In some cases these neurones have been shown by antidromic activation to relay appropriately within the IML, and receive afferent inputs that are important in the regulation of the cardiovascular system (see Gebber, 1990, for review). Equally their activation by electrical or chemical means evokes changes in cardiorespiratory activity. The relative importance of each subset for the maintenance and regulation of the rhythm of sympathetic neurones in the IML remains to be resolved. However, each of these varying subsets of neurones is implicated in other behavioural responses – to pain, in the sleep–wakefulness cycles, affective behaviour, etc. – so that it is likely that the importance of different groups of neurones varies with both state and behaviour, while all provide an overall level of excitatory or inhibitory drive that is integrated moment by moment within the IML.

Vagal preganglionic neurones controlling the heart

The vagal supply is known to be responsible for the heart's major chronotropic regulation. While the physiological role of vagal efferent fibres in exerting negative chronotropic effects has been fully established, the location and properties of the somata of these vagal neurones remained unresolved until work in my laboratory initiated with Robin McAllen (McAllen & Spyer, 1976, 1978*a*,*b*) showed that in the cat, and subsequently in the rabbit also (Jordan, Khalid, Schneiderman & Spyer, 1982), vagal preganglionic motoneurones with the appropriate properties for a role in cardiac control were localized within the nucleus ambiguus (NA) (Figs 4 and 5). This was achieved using electrophysiological approaches (Fig. 5*A*) and has subsequently been confirmed using a range of anatomical tracing techniques

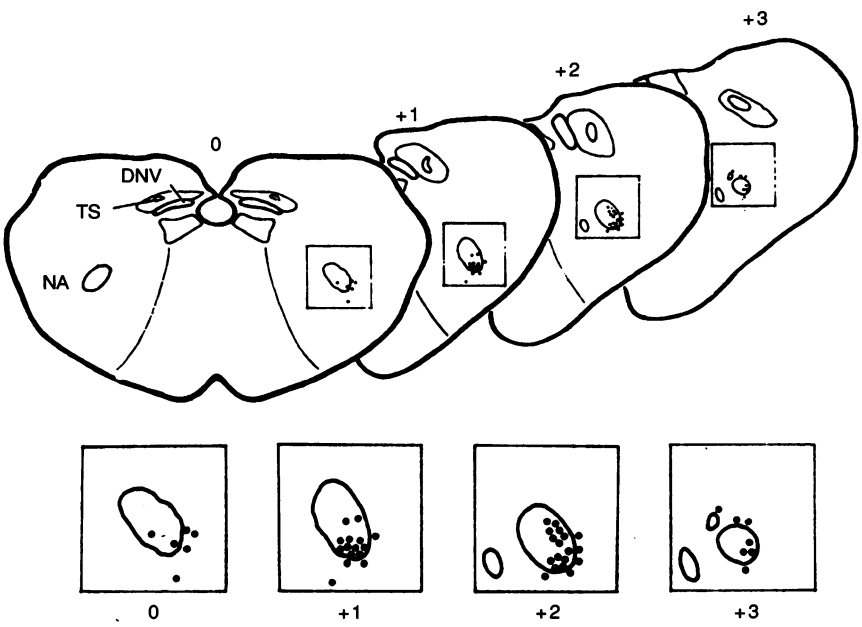

Figure 4. Location of preganglionic vagal cardiac motoneurones
The positions of forty-six cardiac efferent neurones are shown on four standard sections of the medulla taken at obex level, and at 1 mm intervals rostrally. Insets, 2 mm square, show details of their relation to the structure of the nucleus ambiguus. Abbreviations: DNV, dorsal motor nucleus of the vagus; NA, nucleus ambiguus; TS, tractus solitarius. (Reproduced from McAllen & Spyer, 1976.)

(see Loewy & Spyer, 1990, for review). In the cat the dorsal vagal motonucleus is the source of the C fibre innervation of the heart (McAllen & Spyer, 1976), but in the rabbit this nucleus provides both B and C fibre innervation. The major phasic regulation of heart rate involves the activation of neurones with B fibre axons (see later). Our initial observations in the cat thus took on major significance, which was enhanced by the anatomical description of these neurones within the external formation of the NA. They have since been shown to be adjacent to those neurones of the RVLM that have been defined as 'premotor' sympathetic neurones (see above). The 'vasomotor' centre had become a true cardiovascular centre closely interrelated with those neurones that generate respiratory activity. Indeed there may be a level of importance in this arrangement, especially in regard to the organization of reflex inputs and central inputs onto these components of cardiorespiratory control (Richter & Spyer 1990).

Returning to the vagal neurones in the NA of the cat, our observations showed them to fire with a cardiac rhythm (Fig. 5*B*) when active, but as the studies were undertaken in anaesthetized, open-chest and hence artificially ventilated cats the level of vagal 'tone' was low. These neurones were, however, excited by the ionophoretic application of excitant amino acids which both activated the neurone and elicited a small but significant fall in heart rate (McAllen & Spyer, 1978*a*). This latter observation is consistent with the relatively small number of vagal preganglionic neurones that have a cardiac function, while the number of postganglionic cardiac neurones is some two orders of magnitude greater, implying a markedly diverged preganglionic innervation of the parasympathetic ganglia. Just as these neurones showed the influence of the arterial baroreceptor input in their discharge, so they were also seen to have an underlying respiratory discharge pattern (McAllen & Spyer 1978*a*; see Fig. 5*C*). They were active in expiration, confirming the abundant literature describing the firing pattern of vagal efferent fibres in the cervical vagus or vagal cardiac branches that were presumed to have a negative chronotropic function (see Spyer, 1981*a*, *b*, for review). This pattern of discharge appears to be a fundamental property of their synaptic control and may have a phylogenetic origin. In elasmobranch fish the heart and the gills are innervated by branchial vagal neurones that control heart rate and the flow of water through the gills respectively (Ballintjin, 1987).

In the mammal a sympathetic innervation is added to the heart, and respiration depends on both cervical and thoracic motoneurones to drive the diaphragm and thoracic muscles. The original design would seem to argue for a close, if not identical, origin for basic respiratory and cardiac control (Richter, Spyer, Gilbey, Lawson, Bainton & Wilhelm, 1991). Detailed analysis of the firing pattern of cardiac vagal motoneurones takes this even further. They fire in stage I of expiration – post-inspiration (Fig. 5*C*) – with a variable

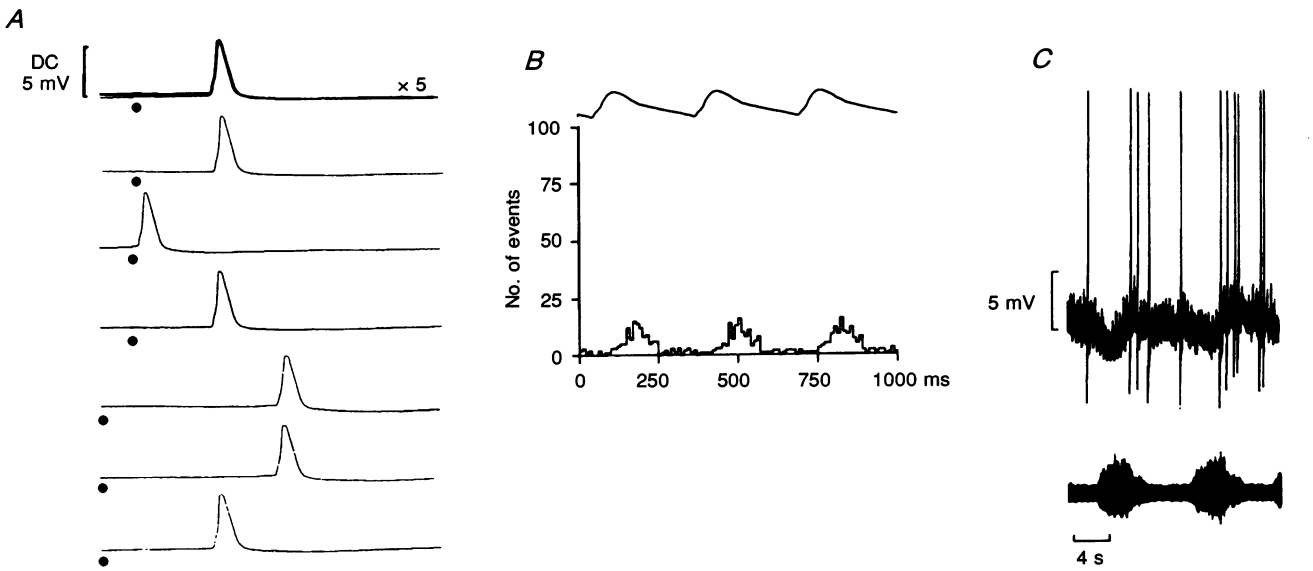

Figure 5. CVM patterns of discharge
A, traces showing CVM patterns of discharge. 1st (upper) trace, stimulating cervical vagus (0·1 ms, 6 V) at 1 Hz. Five superimposed sweeps. 2–4th traces, as above but three consecutive sweeps illustrating the collision of an orthodromic spike, in the middle trace, with the antidromic spike. 5–7th traces, stimulation of the cardiac branches of the right vagus (0·1 ms, 3 V). Three consecutive sweeps, collision with orthodromic spike in lower trace. Stimulus marked by ●. *B*, lower trace, electrocardiogram (ECG)-triggered histogram of on-going discharge of the CVM identified in *A* (100 sweeps, 10 ms bins); upper trace, femoral arterial waveform averaged over the same time course and triggered from ECG (100 sweeps). *C*, intracellular recording from a CVM showing changes in membrane potential in relation to phrenic nerve activity. Upper trace, high-gain DC recording of membrane potential; lower trace, phrenic nerve activity. (Traces taken from Gilbey *et al.* 1984.)

discharge in stage II expiration, and are inhibited during inspiration (Gilbey, Jordan, Richter & Spyer, 1984). Their discharge is thus identical to a class of medullary respiratory neurones – the post-inhibitory neurones – that are an integral component of the respiratory rhythm-generating network (Richter & Spyer, 1990). The only distinguishing feature is that cardiac vagal motoneurones (CVMs) innervate the postganglionic neurones supplying the heart while post-inspiratory neurones generally are propriobulbar (there are, however, notable exceptions in some species). Few post-inspiratory neurones in the cat have defined outputs, although the actions of post-inspiration are evident in the activity of numerous respiratory and autonomic outflows (see Richter & Spyer, 1990). Inspiratory inhibition of CVM activity ensures that any stimulus that enhances inspiration actively increases heart rate while inputs, peripheral or central, that suppress ventilation or prolong expiration lower heart rate (see Daly, 1985). This has crucial significance for cardiorespiratory homeostasis and will be important in subsequent considerations of CNS control of cardiovascular function.

These considerations have concentrated on the role of vagal cardiac neurones with B fibre axons. My colleagues David Jordan and James Jones are, however, studying the role of cardiac vagal C fibre axons. As was first shown in the work of Brown & Eccles (1934*a*, *b*), when stimulated their action is also to slow the heart but with a different time course to those neurones with B fibre axons. They have a somewhat smaller effect and this builds up slowly. It seems that they exert a tonic influence on the heart, unlike the more phasic action of the B fibre input (see above), and may have a more restricted afferent input, being sensitive largely to unmyelinated vagal afferent inputs but not to baroreceptor and chemoreceptor afferent inputs. The functional significance of this dual preganglionic innervation remains to be resolved, as does the pharmacology of their interactions within the parasympathetic ganglia.

Afferent inputs to the brainstem

The majority of cardiovascular and respiratory afferents relay to the CNS via the vagus and glossopharyngeal nerves and terminate within the nucleus tractus solitarii (NTS)

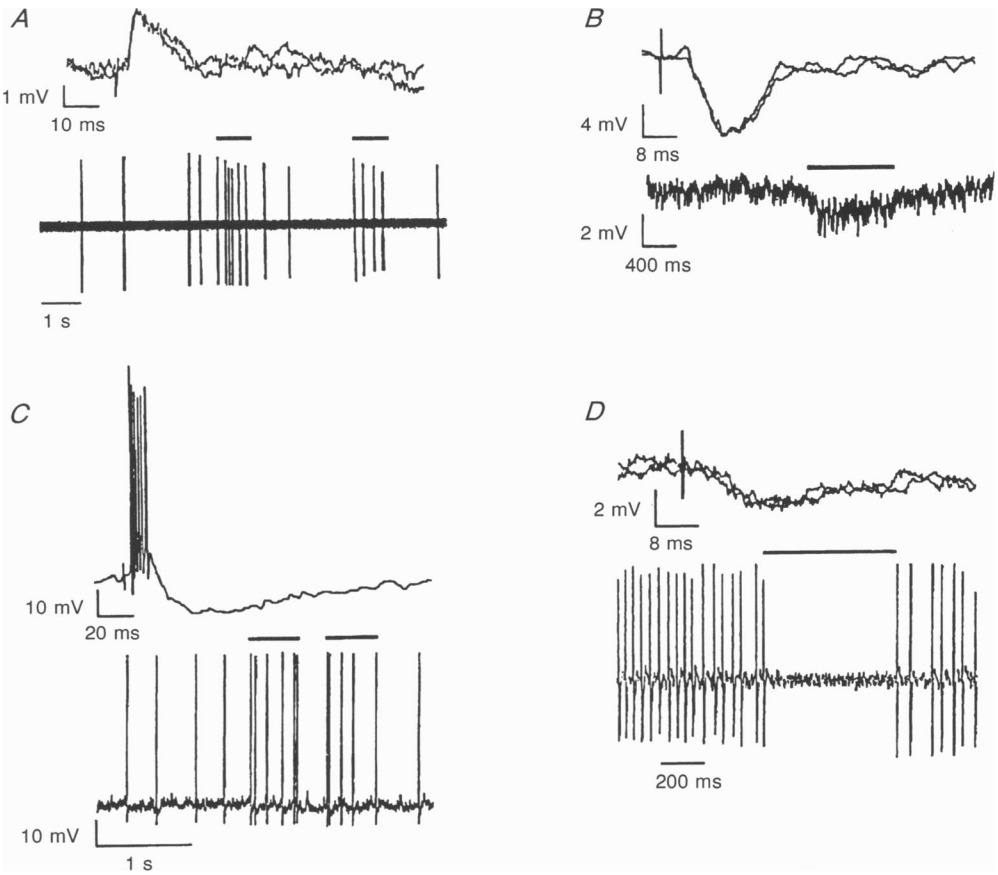

Figure 6. Composite illustration of the responses of NTS neurones to baroreceptor stimulation
Upper traces, intracellular recordings of the response to sinus nerve (SN) stimulation; lower traces, the response of the neurone to inflation of a balloon-tipped catheter in the ipsilateral carotid sinus. Inflation indicated by horizontal bar. *A*, neurone showing IPSP to SN stimulation. *B* and *C*, neurones showing EPSP–IPSP to SN stimulation. *D*, neurone showing EPSP to SN stimulation. (Traces taken from Mifflin, Spyer & Withington-Wray, 1988*a*, *b*.) See text for further details.

(Jordan & Spyer, 1986; Loewy, 1990). This nucleus functions as the primary site of reflex integration and is also richly innervated from regions of the CNS concerned directly or indirectly with cardiorespiratory control (Loewy, 1990). The nucleus is also concerned with other aspects of autonomic function but there appears to be a certain level of organization of function within the nucleus, even though this may not be represented within discrete cytoarchitectonic boundaries. As yet we do not have the detailed correlated anatomical and physiological data that are available for the dorsal horn of the spinal cord (Brown, 1981) to which the NTS is often compared but there are reasonable inferences about the spatial organization of inputs and a detailed knowledge of the physiological properties of the recipient neurones. Indeed the physiological, biophysical and pharmacological properties of NTS neurones are amenable to detailed investigation using *in vitro* slice preparations of the medulla (see, amongst others, Champagnat, Denavit-Saubie, Grant & Shen, 1986; Champagnat, Jacquin & Richter, 1986; Brooks, Glaum, Miller & Spyer, 1992). Such studies have complemented the data derived from *in vivo* studies and provided an explanation for many of the features of interaction that have been identified *in vivo*.

Particular attention has focused on the organization of the arterial baroreceptor and chemoreceptor reflex inputs to the NTS, as well as the inputs from pulmonary and airway receptors. The detailed pattern of innervation has been derived largely from anatomical studies, although antidromic mapping techniques have provided the most relevant data with regard to functional organization (Jordan & Spyer, 1986). In this context the central pattern of projection and synaptic actions of slowly adapting lung stretch afferents have been investigated to greatest effect (Averill, Cameron & Berger, 1984; Backman *et al.* 1984; Kalia & Richter, 1985*a*, *b*). In the case of the arterial baroreceptor input there has grown a belief based largely on indirect morphological studies that the dorsomedial portion of the NTS at a level just rostral to the obex is the site of the second-order baroreceptor neurones (see Seller & Illert, 1969; Czachurski, Dembowsky, Seller, Nobiling & Taugner, 1988). Antidromic mapping studies, and more detailed electrophysiological analysis of the pattern of response of NTS neurones to electrical stimulation of the sinus nerve (SN) and natural baroreceptor stimulation, have given a rather different picture. It appears that baroreceptor afferents project to the dorsal areas of the medial and lateral divisions of the NTS primarily from the level of the obex rostrally, with a significant input to more lateral components of the NTS, at least in the cat (Donoghue, Felder, Jordan & Spyer, 1984; Mifflin *et al.* 1988*a*, *b*). Neuronal recordings have lent support to the notion of a dorsal strip with many cells showing a powerful and short latency response to SN stimulation, but this strip extends into the lateral and ventrolateral components of the NTS (Spyer, 1990). The latencies of evoked EPSPs, rise times and often complex configuration suggest that a significant proportion of these neurones receive both mono- and polysynaptic SN inputs.

It appears that there is a circuitry of interacting interneurones within the NTS that processes this input. On the basis of the latency and pattern of evoked EPSPs or IPSPs (or a combination of the two) and the pattern of response to specific baroreceptor input, the potential connections mediating these observed responses can be summarized (see Fig. 7). This organization becomes particularly relevant when the actions of descending inputs to the NTS from the hypothalamus are considered (see later).

The arterial chemoreceptor input has been mapped electrophysiologically in an equivalent manner to baroreceptors. Chemoreceptor afferent fibres appear to terminate preferentially in the commissural NTS at the level of, and behind, the obex with a significant input into the medial nucleus and a sparse input to the ventrolateral subnucleus (Donoghue *et al.* 1984). Neurones in these regions have been shown to receive a monosynaptic excitatory input on SN stimulation, and to be excited by natural chemoreceptor stimulation (Spyer, Izzo, Lin, Paton, Silva-Carvalho & Richter, 1990; Mifflin, 1992). There is some evidence to indicate that approximately 25% of NTS neurones receive convergent excitatory inputs from the arterial baroreceptors and chemoreceptors, although these have not been observed in studies

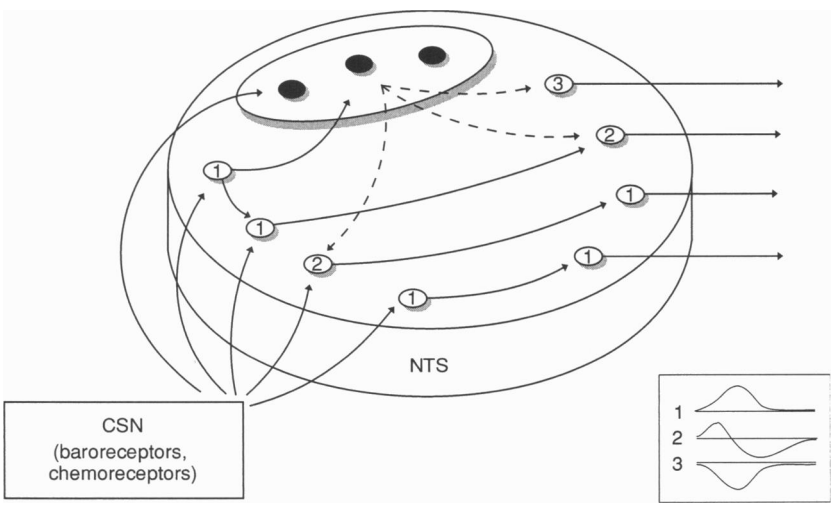

Figure 7. Schematic diagram of connections within the nucleus tractus solitarii (NTS) that mediate arterial baroreceptor and chemoreceptor inputs
Effects elicited in different neurones on sinus nerve stimulation (CSN) – EPSP (1); EPSP–IPSP (2) and IPSP (3) – are shown in the inset. Excitatory connections shown as continuous lines; inhibitory connections as dashed lines. Neurones are numbered according to pattern of response, except intrinsic GABA-containing neurones which are shown as filled ovals. Further details in text.

from this laboratory (Mifflin, 1992). Some NTS neurones that display an EPSP–IPSP response to SN stimulation receive an exclusively inhibitory input from the arterial baroreceptors so that the excitatory component must be expected to result from activation of chemoreceptor inputs (see Fig. 6). In this regard we now believe that the pattern of response is in part determined by the activation of a group of intrinsic GABA-containing neurones which are localized within the parvocellular portion of the NTS (see Izzo, Sykes & Spyer, 1992; Brooks, Izzo & Spyer, 1993, for review). Equivalent patterns of response can be evoked on stimulation of the tractus solitarius in the *in vitro* slice preparation, making it reasonable to assume that the inhibitory components of SN-evoked responses result from the direct, or indirect, activation of these GABA-containing neurones, since primary afferents do not evoke monosynaptic inhibitory actions (Brooks *et al.* 1993). The potential connections mediating these various patterns of response are summarized in Fig. 7.

The inhibitory role of GABA in the NTS has been a major focus of investigation in the last few years. The GABAergic innervation of the NTS in the cat, and also the rat, appears to be relatively homogeneous (Izzo *et al.* 1992; Brooks *et al.* 1993). Neurones in all the main divisions of the nucleus have been shown to be contacted by GABA-containing terminals and these synaptic contacts are made primarily with dendritic spines and shafts, some onto the neuronal soma but virtually none onto axons. These connections can be dissected pharmacologically in both *in vivo* and *in vitro* studies and are accounted for largely by the action of GABA at $GABA_A$ receptors (i.e. those that are antagonized by bicuculline) (Jordan, Mifflin & Spyer, 1988; Brooks *et al.* 1992). The absence of a substrate for axo-axonal actions is reasonable with regard to the known interactions of both baroreceptor and chemoreceptor inputs, but vagal afferents are known to interact presynaptically (Jordan & Spyer, 1986). Axo-axonal connections involving GABA have been identified in the ventrolateral subnucleus in cat which is considered to contain one of the main populations of respiratory neurones (Lipski, Waldvogel, Pilowski & Jiang, 1990).

In the rat medullary slice the action of GABA acting at $GABA_B$ receptors has been identified (Brooks *et al.* 1992). It has a postsynaptic hyperpolarizing action when applied to the bath in the micromolar range but at nanomolar levels acts presynaptically to depress both EPSPs and IPSPs. In this way the absence of axo-axonal synaptic specializations may be irrelevant with regard to presynaptic actions of GABA. Thus GABA release can be expected to elicit subtle changes in synaptic action and the control of GABA-containing neurones of the NTS must represent a major factor in the integration of cardiorespiratory reflexes but will also provide CNS structures with a means to modify the performance of these reflexes.

Efferent projections of the NTS

The NTS has well-defined, and often reciprocal, connections with numerous areas of the brainstem as well as to both forebrain structures and the spinal cord (see Loewy, 1990, for detailed review). In the context of cardiovascular control its relationships with cell groups in the lower medulla may be of primary significance. The NA and DVN (dorsal motor nucleus of the vagus) both receive marked innervations from the NTS, and in the context of CVMs the direct projection from the NTS to those in the NA is particularly revealing (Deuchars & Izzo, 1991). There is a marked lateralization of the output from the NTS and it appears that the ventrolateral medulla is a major target for this innervation (see Fig. 8). This will include the vagal and respiratory neurones of the NA and the adjacent RVLM, and also the more laterally displaced 5-HT-containing neurones. A marked innervation from the NTS has also been shown caudally in the region of the A1 group of catecholamine-containing neurones first implicated in cardiovascular control by Coote & Macleod (1974*a*, *b*), but extending to the NA and now part of a region termed the caudal ventrolateral medulla (CVLM) (Dampney, 1994). Neurones in the CVLM are considered to receive a baroreceptor input and to relay rostrally to control the activity of the RVLM, although also having a marked ascending influence extending as far as the hypothalamus. The RVLM receives a direct innervation from the NTS but there is a consensus that this mediates chemoreceptor influences (Guyenet, 1990; Dampney, 1994). The location of the GABA-containing neurones that exert the baroreceptor-mediated inhibitory action on RVLM neurones remains to be identified, and whether these are targets for other inhibitory inputs is also subject to verification.

Evidence exists for reciprocal relationships between the ventrolateral medulla and the NTS (Loewy, 1990), and the more rostrally placed Bötzinger complex at the pontine border has a marked input to the NTS that is considered to have a fundamental role in respiratory control (Merrill, Lipski, Kubin & Fedorko, 1983). The interrelationships between the pontine parabrachial complex and the NTS are particularly important given that this complex also has important interconnections with the ventrolateral medulla (Loewy, 1990). From current investigations it is emerging that there are several ascending pathways from the NTS to the pons, midbrain, hypothalamus and forebrain that are matched by descending connections involving these same nuclear groupings (Loewy, 1990).

The physiological role of these connections is as yet unresolved but the effects mediated by connections restricted to the lower brainstem have been analysed in some depth using *in vivo* experiments in cat, rabbit and rat. Remarkably a considerable portion of this circuitry is retained in the *in vitro* medullary slice and this is allowing even more detailed correlated physiological, morphological and pharmacological studies to be attempted. With regard to our primary objective of determining the basic circuits responsible for cardiovascular control, it is clear that CVMs receive a monosynaptic input from the NTS, making it possible to envisage a relatively simple basic reflex pathway for baroreceptor–vagal control of the heart. This substantiates the measured latency of the reflex which indicated both a short and longer latency component in the cat (McAllen & Spyer, 1978*b*). The latency of the inhibitory effects of

baroreceptor stimulation on RVLM neurones is similar to these timings (McAllen, 1987), indicating that relatively slowly conducting fibres are involved and that a considerable portion of the reflex input may result from more long-circuited connections, presumably over the general reciprocal network revealed anatomically (implied in the connections between Figs 3 and 8). The nature of the neurotransmitters that are involved in these synaptic mechanisms is unknown, although there is sufficient general information available to support the involvement of glutamate influences on all components of the medullary network, but since the NTS is rich in peptide-containing neurones and terminals and as most putative transmitters and modulators are present in the medulla the alternatives are plenty. In the case of CVMs within the NA in cat and rat evidence exists for the presence of substance P (Deuchars, Spyer & Izzo, 1992), 5-HT (Izzo, Deuchars & Spyer, 1993), GABA (Maqbool, Batten & McWilliam, 1991), glutamate and enkephalins in the synaptic contacts made on them. Similar data are available for RVLM neurones (see Dampney, 1994, for review). As we have seen, within the NTS, GABA is the major inhibitory neurotransmitter but the role of glycine and other inhibitory modulators remains to be investigated.

The medullary raphe complex, particularly the caudally placed raphe pallidus and obscurus, also receives a marked innervation from the NTS and may well provide a major portion of the serotinergic innervation of the NTS, NA and RVLM. While the direct role of the raphe complex in mediating baroreceptor reflex responses has not been substantiated there is excellent evidence, both morphological and physiological, that it provides a major innervation of the sympathetic preganglionic neurones of the IML (see above). Raphe neurones show evidence of both respiratory and cardiovascular rhythms in their discharge (Morrison & Gebber, 1982; Gebber, 1990; Lindsey, Hernandez, Morris & Shannon, 1992*a*; Lindsey, Hernandez, Morris, Shannon & Gerstin, 1992*b*; Marks & Gilbey, 1992; Zhou, Futuro-Neto & Gilbey, 1993) and are involved in other behaviour such as sleep and the response to pain. Accordingly they may play a pivotal role in modulating reflex function and in determining the level of excitability of the autonomic preganglionic neurones.

Descending control of cardiovascular function

The previous sections have revealed that much of the basic structure for the reflex control of the cardiovascular system is contained within the medulla (see Fig. 8). This will provide a rudimentary level of regulation, but for integrated and behaviourally significant responses the reciprocal connections that have been shown to exist between medulla, pons, midbrain and hypothalamus are essential. The functional role of suprabulbar structures in cardiovascular control is more difficult to assess, however, since the ablation and stimulation studies that have been undertaken provide, at best, a crude assessment of their role. To obtain a more structured understanding of the action of suprabulbar areas in cardiovascular regulation it has proved fruitful to take advantage of the specific actions of the CNS in mediating the responses associated with affective behaviour. Evidence indicates that subcortical regions – including the

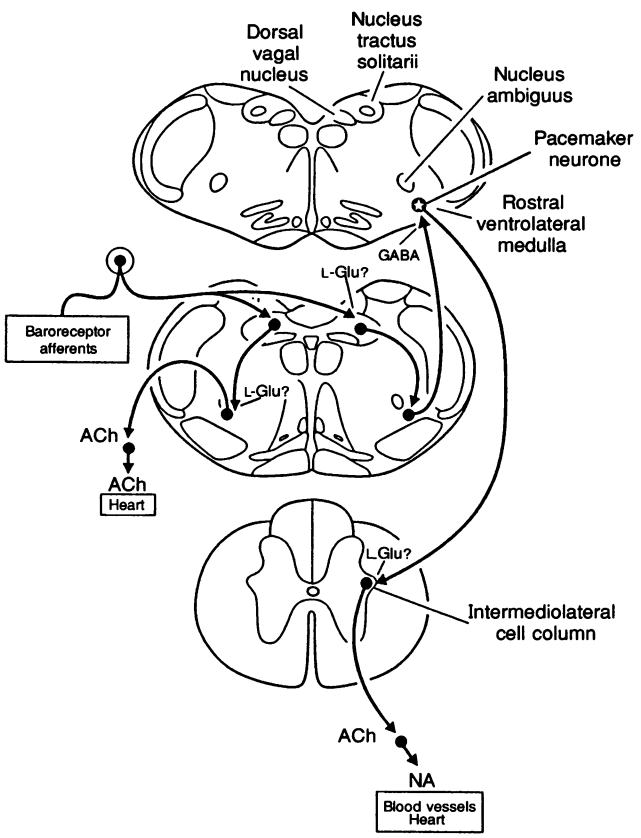

Figure 8. A model of the neural circuitry involved in the baroreflex
Incoming baroreceptor afferents terminate in the nucleus tractus solitarii. These first-order neurones have their cell bodies in the ganglia of the IXth and Xth cranial nerves. The transmitter involved is unknown, but may be L-glutamate or a related chemical. Second-order neurones in the nucleus tractus solitarii project to two sites in the medulla oblongata: cardiac preganglionic neurones (shown on left) and GABA-containing neurones in the region near the nucleus ambiguus (shown on right). The latter are thought to project to pacemaker neurones in the rostral ventrolateral medulla. The pacemaker neurones may project directly to the sympathetic preganglionic neurones and use L-glutamate or a related chemical as a transmitter. The transmitters used at each central synapse remain speculative because the full requirements needed for proof that a particular chemical is acting as a transmitter have not been fulfilled. (Reproduced with permission from Guyenet, 1990.)

central nucleus of the amygdala (CEN) and restricted regions of the hypothalamus – are essential in patterning the behavioural and underlying cardiovascular, and the autonomic, features of these responses (Hilton, 1966; Jordan, 1990). Similarly, during exercise sustained motor activity would be impossible without significant alterations in cardiovascular and respiratory activity. Both examples fit well with Cannon's precepts of the role of the autonomic nervous system but spread much wider in implying that behavioural programmes involve a distinctive patterning of autonomic function. There must therefore be a repertoire of cardiovascular patterns of response appropriate for behaviours, but the expression of these will be modulated continually by reflex inputs in order to maintain cardiorespiratory homeostasis. There are also indications that cardiovascular and respiratory reflex inputs can modulate behaviour, and I believe that we now have sufficient evidence to propose that there is also a reciprocal behavioural modification of reflex function. Chemoreceptor inputs are able to elicit the defence reaction (Marshall, 1977), and conversely the hypothalamus is able to facilitate the chemoreceptor reflex (Hilton & Joels, 1965; Silva-Carvalho, Dawid-Milner, Goldsmith & Spyer, 1993). In contrast baroreceptor inputs have been shown to inhibit the sham rage of the high-decerebrate cat (Bartorelli, Bizzi, Libretti & Zanchetti, 1960). Together these observations argue for a dynamic interaction between central drives and reflex inputs in adjusting cardiorespiratory control to subserve behaviour while maintaining homeostasis. This dynamic interaction can be considered to provide a compact pattern of organization – collaterals from descending 'behavioural' or 'motor' pathways having access to the autonomic reflex circuits whose sensitivities are themselves set by the prevailing level of reflex inputs. To test this hypothesis we have investigated the mechanisms by which activation of the 'defence' areas of the hypothalamus and amygdala elicit their characteristic changes in cardiovascular activity.

Stimulating electrically within the hypothalamus, and in the CEN, in the appropriately anaesthetized animal elicits a cardiovascular pattern of response that involves an increase in arterial blood pressure and heart rate (Fig. 9). This contributes to a rise in cardiac output, and the patterning of

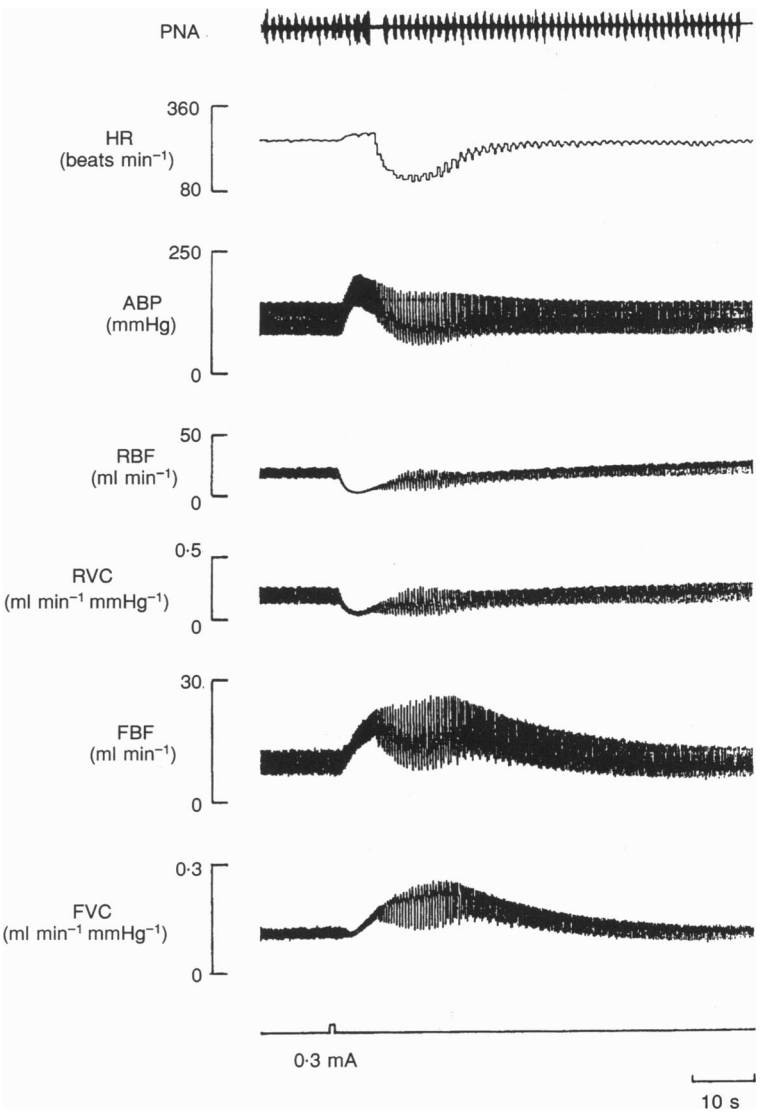

Figure 9. Cardiovascular and respiratory responses to electrical stimulation in the perifornical region of the hypothalamus of the althesin-anaesthetized cat
The stimulus was a 4 s train of 0·5 ms pulses delivered at 100 Hz, initiated at the time shown in the lower trace on the polygraph record shown on the left-hand side. Traces from top to bottom: PNA, phrenic nerve activity; HR, heart rate; ABP, arterial blood pressure; RBF, renal blood flow; RVC, renal vascular conductance; FBF, femoral blood flow; FVC, femoral vascular conductance. RBF and FBF were recorded using electromagnetic flow probes. (Reproduced with permission from Spyer, 1989.)

sympathetic efferent activity leads to a diversion of this to the skeletal muscles at the expense of the internal organs (Hilton, 1966; Timms, 1981). While there is a very generalized rise in sympathetic vasoconstrictor activity, the vascular beds of skeletal muscle dilate as a consequence of the activation of a cholinergic vasodilator innervation, the release of catecholamines from the adrenal medulla into the circulation and a lowering sympathetic vasoconstrictor tone specifically within the muscle bed (Fig. 9). Accompanying these changes respiratory minute volume increases and other autonomic manifestations include piloerection, retraction of the nictitating membrane and pupillodilatation. The simultaneous rise of blood pressure and heart rate suggested to Hilton (1966) that the baroreceptor reflex was blocked during the defence reaction and subsequent studies by Coote, Hilton & Zbrozyna (1973) indicated that this involved a central modification of reflex function. On the basis of the evidence that there are numerous descending pathways from the diencephalon and midbrain to the NTS, we have tested the possibility that this resetting of the baroreceptor reflex might involve a synaptic action at the level of the integrative circuitry with the NTS described earlier.

Intracellular recordings in the NTS have shown that a conditioning stimulus applied to the hypothalamic defence area elicits IPSPs in those NTS neurones that are excited by sinus nerve stimulation and by natural baroreceptor activation (Mifflin, Withington-Wray & Spyer, 1988*b*). The SN-evoked EPSPs are shunted as a consequence of the resultant fall in membrane input resistance (Fig. 10). This effect can totally suppress the action of the baroreceptor input. In confirmation of the proposed interneuronal structure of the NTS described earlier (Fig. 7) other neurones that are excited polysynaptically from the SN have often been shown to have this input blocked as a consequence of disfacilitation. Equally NTS neurones that are inhibited by SN and baroreceptor stimulation are often excited by hypothalamic stimulation. Interestingly, and in accord with the presented scheme of organization (Fig. 11), the inhibitory effects of SN stimulation within the NTS are also blocked by hypothalamic stimulation, indicating that hypothalamically evoked inhibitory actions are directed especially to those NTS neurones that are monosynaptically excited by baroreceptor inputs (Mifflin *et al.* 1988*b*). These actions of hypothalamic stimulation involve GABA acting at $GABA_A$ receptors since the inhibitory actions of hypothalamic stimulation can be antagonized by the ionophoretic application of bicuculline (Fig. 12) but not strychnine. The descending inputs to the NTS from the hypothalamus, midbrain and pons do not involve GABA-containing fibres (Izzo *et al.* 1992). It thus appears that the intrinsic NTS GABA-containing neurones must mediate these effects and so be the targets for these descending inputs. Certainly preliminary studies have identified neurones in the NTS with the appropriate physiological properties as representing these interneurones but these have not as yet been shown to contain GABA (Mifflin *et al.* 1988*b*; Silva-Carvalho *et al.* 1993).

At one time it was believed that this form of reflex 'gating' was only elicited by stimulation of the hypothalamic defence area and so was a distinctive feature of affective behaviour. More recent studies indicate that this is a more general

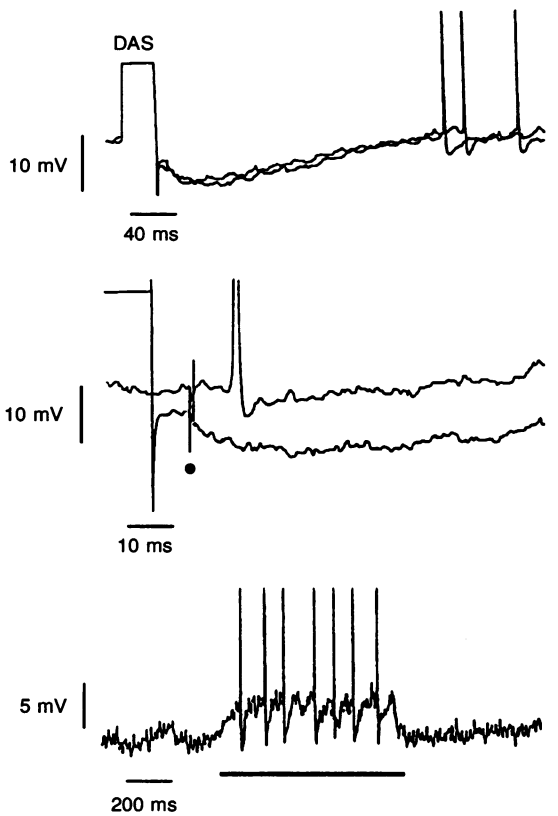

Figure 10.
Bottom trace, intracellular recording from an NTS neurone that received a powerful baroreceptor input. The horizontal bar indicates the period when the ipsilateral carotid sinus was inflated through a balloon-tipped catheter. Middle traces, the response of this neurone to electrical stimulation of the sinus nerve alone, the lower of the two traces showing the response after a conditioning stimulus delivered to the hypothalamic defence area. Note the hyperpolarization and absence of the evoked spike response. Upper trace, two superimposed sweeps illustrating the time course of the defence area evoked hyperpolarization. Stimulation to the hypothalamic defence area involved a 30 ms, 500 Hz burst of stimuli (DAS). (Reproduced from Mifflin *et al.* 1988*b*.)

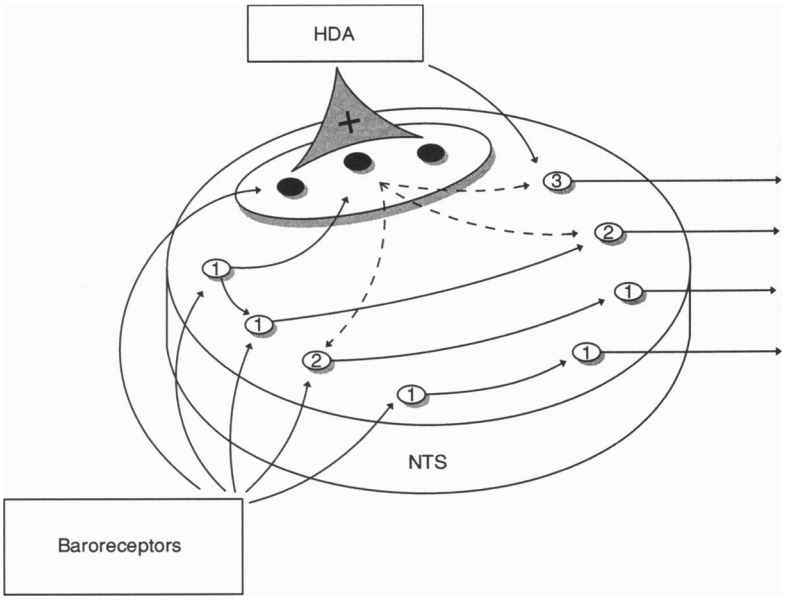

Figure 11.
Schematic diagram illustrating the synaptic connections mediating hypothalamic defence area (HDA) actions within the NTS. Details as in Fig. 7. Further details in text.

property of the CNS. The uvula cortex of the posterior cerebellar vermis has been shown to affect both cardiovascular and respiratory systems when activated (Bradley, Ghelarducci & Spyer, 1991; Paton & Spyer, 1992). The pattern of response is qualitatively modified by the presence of anaesthetic agents (Fig. 13). Its behavioural significance is as yet unresolved, although in the unanaesthetized and decerebrate animal the pattern of cardiorespiratory response is not dissimilar to that evoked by stimulation in the hypothalamic defence area. This pattern of response is also dependent on the activation of GABA interneurones in the NTS and involves a modulation of baroreceptor function (Paton, Silva-Carvalho, Goldsmith & Spyer, 1990). In the exercise reflex, in which this region of the cerebellum may also play a part, the baroreceptor reflex appears to be modulated and again there are indications that this involves a GABAergic action within the NTS (McWilliam, Yang & Chen, 1991). While the role of GABA acting at $GABA_A$ receptors is implicated in all these physiological actions, the potential action at $GABA_B$ receptors that have been illustrated so markedly *in vitro* (see above) have received no attention as yet. It is, however, known that $GABA_B$ receptor agonists when injected *in vivo* into the NTS elicit rises in arterial blood pressure (Sved, Tsukamoto & Sved, 1993).

These data imply that GABAergic mechanisms in the NTS may play essential roles in the integrative interactions

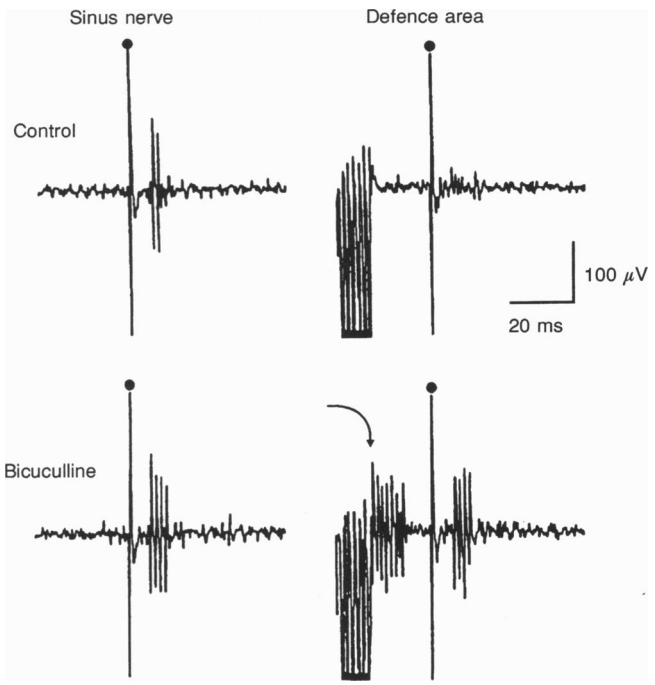

Figure 12. The effect of bicuculline on SN-evoked activity and its inhibition by HDA stimulation in NTS neurones
Each panel shows single oscilloscope sweeps of activity evoked by a SN stimulus (0·1 ms, given at ●) before (above) and during (below) application of the antagonist. On the right the SN stimuli are preceded by a stimulus train to the HDA (five pulses, 1 ms, 500 Hz) ending 20 ms before the SN stimulus. Arrow indicates excitatory response evoked from the hypothalamus in presence of bicuculline (43 nA). (Reproduced from Jordan, Mifflin & Spyer, 1988.)

between reflex inputs and the effects of activating regions of the CNS. Indeed there may be subtle interactions as well as the more gross effects from which so much experimental information has been derived. Furthermore, these are not the only interactions that take place within the NTS. As demonstrated in the experiments in which GABA was shown to mediate hypothalamic control of the baroreceptor reflex, when this action was antagonized an additional interaction became evident. Hypothalamic stimulation then evoked a short latency excitation of those NTS neurones that were excited by the baroreceptor input (Jordan *et al.* 1988; see Fig. 12). Similar facilitatory interactions have been observed in the absence of $GABA_A$ receptor antagonists in the rabbit when the CEN was stimulated (Cox, Jordan, Moruzzi, Schwaber, Spyer & Turner, 1985; see Fig. 14) and these observations correlated well with the fact that CEN activation in the conscious rabbit evokes a 'playing dead' response that includes bradycardia and systemic hypotension (Applegate, Kapp, Underwood & McNall, 1983). Presumably the individual mammal has the ability to produce the complete repertoire of behavioural responses to threatening stimuli from fear, flight or rage to 'playing dead'. These responses are organized within the same CNS structures and electrical stimuli when applied do not discriminate between them (Jordan, 1990). In the cat the aggressive features of behaviour are better developed but cats do 'play dead' or adopt submissive postures in certain circumstances. Even a

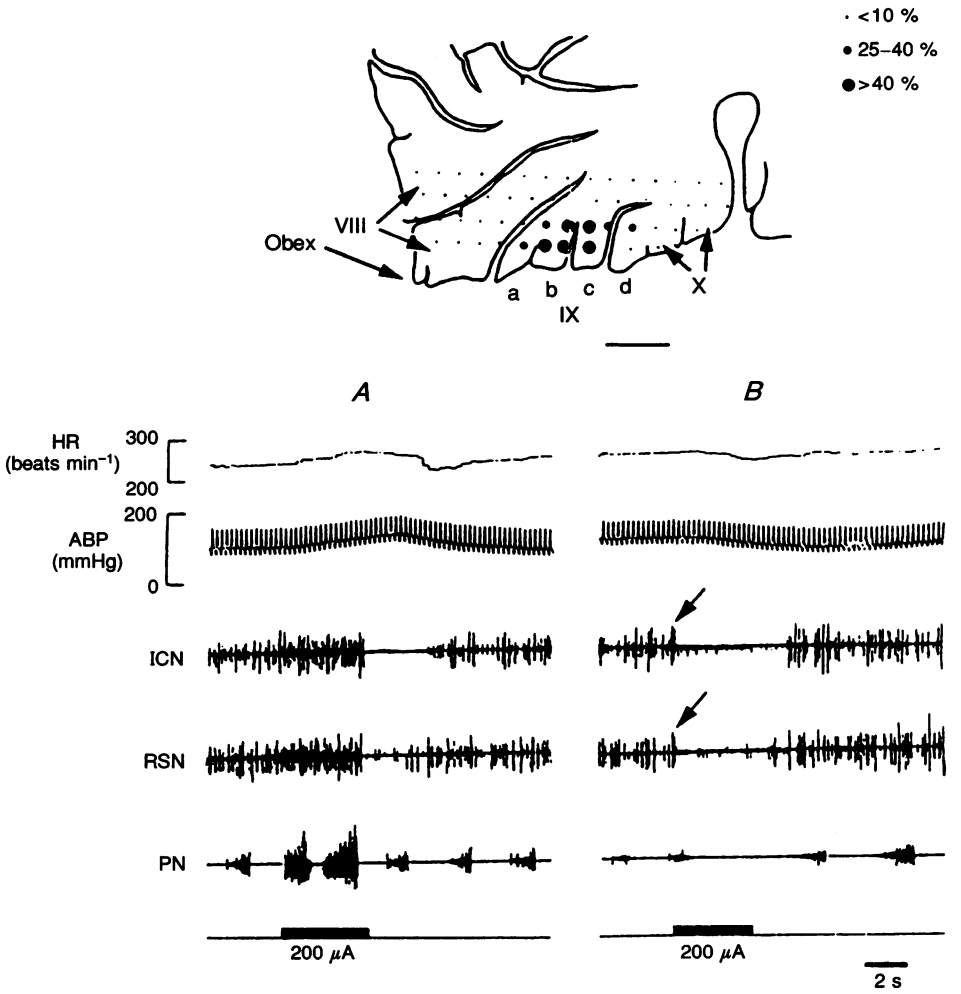

Figure 13.
Top, representative midline sagittal section of cat posterior cerebellar vermis showing that the responsive region was localized to sublobules b and c of the uvula (lobule IX). Symbols indicate stimulation sites within lobules VII–X. The percentage change in heart rate (HR) and arterial blood pressure (ABP) evoked is indicated by symbol size. Bar = 1 mm. Bottom, comparison of cardiovascular and phrenic nerve (PN) changes elicited from the uvula in decerebrate (*A*) and anaesthetized decerebrate (*B*) cat during electrical stimulation (100 Hz, 0·1 ms). Qualitatively similar responses were observed during intracortical microinjection of glutamate. Notice the transient burst in discharge in the inferior cardiac nerve (ICN) and renal sympathetic nerve (RSN) at onset of stimulus in anaesthetized animal (at arrows; see text for explanation). (Reproduced with permission from Paton & Spyer, 1992.)

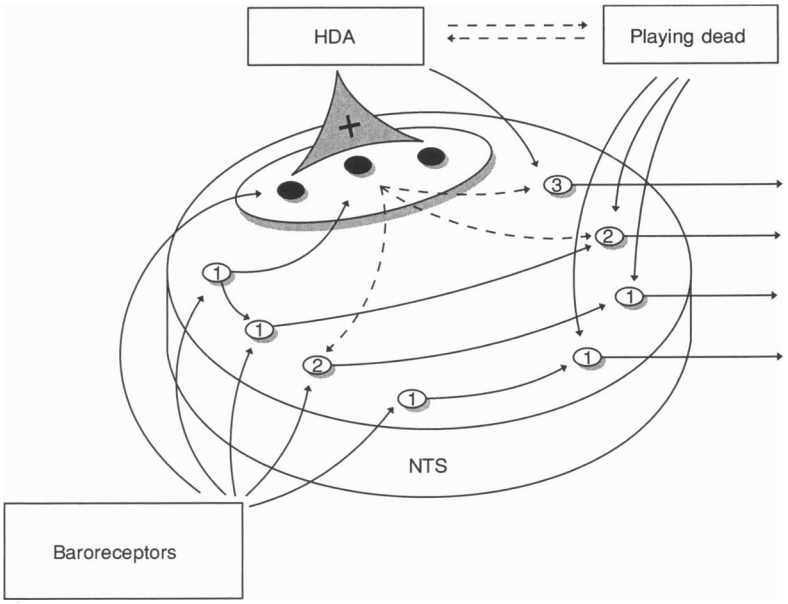

Figure 14.
Schematic diagram illustrating the interplay of behavioural drives within the hypothalamus and NTS that influence circulation. Details as in Figs 7 and 11.

rabbit can express aggressive behaviours, if only to other rabbits! This suggests that the matrix for interaction in affective behaviour is indeed complex and future interest will concern the identification of the chemical code of the multiple descending inputs to the NTS subserving these behaviours (see Fig. 14).

Descending inputs also access the 'premotor' sympathetic and vagal neurones, implying that autonomic changes are induced through the activation of specific and rather stereotyped connections. These descending pathways also affect the respiratory neurones of the medulla in a parallel manner. Accordingly the respiratory patterning of

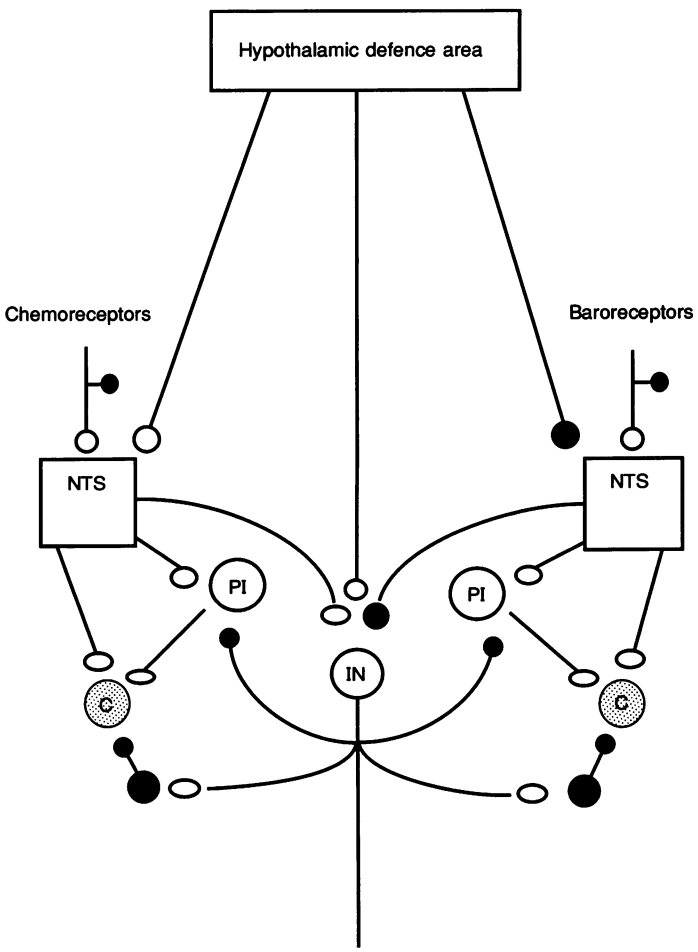

Figure 15.
Schematic diagram of the pathways and synaptic interactions whereby the hypothalamic defence area affects the arterial chemoreceptor (left side) and arterial baroreceptor (right side) reflex control of the vagal outflow to the heart. Excitatory connections, ——○; inhibitory connections, ——●; although these are not necessarily direct monosynaptic action (see also Figs 10 and 13 and the text for further details). C, cardiac vagal motoneurones in the NA; IN, inspiratory neurones; NTS, nucleus tractus solitarii; PI, post-inspiratory (or stage 1 expiratory) neurones.

'premotor' sympathetic neurones and vagal cardiomotor neurones will alter in each situation. In the case of cardiac vagal motoneurones, during the defence reaction this excitatory input from the arterial baroreceptors will be reduced by the interactions within the NTS (disfacilitation); they will receive a direct inhibitory input from the hypothalamus that is mediated by GABA (Spyer, 1984) and they will be inhibited by the concerted activation of inspiratory neurones (see Fig. 15 and Spyer, 1990). Chemoreceptor activation similarly intensifies inspiratory-related inhibitory inputs onto CVMs, and facilitates the defence areas and so provokes a powerful tachycardia (Fig. 15). Conversely inputs derived reflexly, or centrally, that arrest respiratory activity lead automatically by disinhibition to an enhanced vagal tone, chemoreceptor activation then provoking a bradycardia. This mechanism takes on profound significance in breath-hold diving (Daly, 1985). These interactions lend emphasis to the physiological significance of the neurophysiological observations that have been made *in vivo* and in reduced *in vitro* preparations regarding the restricted basic circuitry of cardiovascular control.

Together, these data indicate that the simplified networks that have been identified within the medulla contain within them a substrate that can generate patterns of activity that are relevant for both homeostasis and the behavioural patterning of autonomic activity. The development of these networks from early life, when reflex control is often refractory, to the situation in the adult is intriguing and amenable to investigation at both the cellular and ultimately the molecular level now that the basic central circuitry has been identified. Adaptations of this control may lead to labile changes in autonomic function. The development of neurogenic hypertension may involve inappropriate modifications of synaptic function within these networks. Adaptations of control can now be studied using a battery of techniques and approaches that were until recently unavailable, but advances will constantly require the interplay of *in vivo* physiological studies and *in vitro* cellular and molecular science.

REFERENCES

Alexander, R. S. (1946). Tonic and reflex functions of medullary sympathetic centers. *Journal of Neurophysiology* **9**, 205–217.

Amendt, K., Czachurski, J., Dembowsky, K. & Seller, H. (1979). Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *Journal of the Autonomic Nervous System* **1**, 103–117.

Applegate, C. D., Kapp, B. S., Underwood, M. D. & McNall, C. L. (1983). Autonomic and somatomotor effects of amygdala central nucleus stimulation in awake rabbits. *Physiology and Behaviour* **31**, 353–360.

Averill, D. B., Cameron, W. E. & Berger, A. J. (1984). Monosynaptic excitation of dorsal medullary respiratory neurones by slow adapting pulmonary stretch receptors. *Journal of Neurophysiology* **52**, 771–785.

Backman, S. B., Anders, C., Ballantyne, D., Rohrig, N., Camerer, H., Mifflin, S., Jordan, D., Dickhaus, H., Spyer, K. M. & Richter, D. W. (1984). Evidence for a monosynaptic connection between slowly adapting pulmonary stretch receptor afferents and inspiratory beta neurones. *Pflügers Archiv* **402**, 129–136.

Bacon, S. J., Zagon A. & Smith, A. D. (1990). Electron microscopic evidence of a monosynaptic pathway between cells in the caudal raphé nuclei and sympathetic preganglionic neurons in the rat spinal cord. *Experimental Brain Research* **79**, 589–602.

Bacon, S. J. & Smith, A. D. (1993). A monosynaptic pathway from an identified vasomotor centre in the medial prefrontal cortex to an autonomic area in the thoracic spinal cord. *Neuroscience* **54**, 719–728.

Bacon, S. J., Zagon A. & Smith, A. D. (1990). Electron microscopic evidence of a monosynaptic pathway between cells in the caudal raphe nuclei and sympathetic preganglionic neurons in the rat spinal cord. *Experimental Brain Research* **79**, 589–602.

Ballintijn, C. M. (1987). Evolution of central nervous control of ventilation in vertebrates. In *Studies in Neuroscience: Neurobiology of the Cardiorespiratory System*, ed. Taylor, E. W., pp. 3–27. Manchester University Press, Manchester.

Barman, S. M. & Gebber, G. L. (1985). Axonal projection patterns of ventrolateral medullospinal sympathoexcitatory neurons. *Journal of Neurophysiology* **53**, 1551–1566.

Bartorelli, C., Bizzi, E., Libretti, A. & Zanchetti, A. (1960). Inhibitory control of sinocarotid pressoceptive afferents on hypothalamic autonomic activity and sham-rage behaviour. *Archives Italienne de Biologie* **98**, 308–326.

Bayliss, W. M. (1923). *The Vasomotor System.* Longman, London.

Bradley, D. J., Ghelarducci, B. & Spyer, K. M. (1991). The role of the posterior cerebellar vermis in cardiovascular control. *Neuroscience Research* **12**, 45–56.

Brooks, P. A., Glaum, S. R., Miller, R. J. & Spyer, K. M. (1992). The actions of baclofen on neurones and synaptic transmission in the nucleus tractus solitarii of the rat *in vitro*. *Journal of Physiology* **457**, 115–129.

Brooks, P. A., Izzo, P. N. & Spyer, K. M. (1993). Brain Stem GABA pathways and the regulation of baroreflex activity. In *Central Neural Mechanisms in Cardiovascular Regulation*, vol. 2, ed. Kunos, G. & Ciriello, J., pp. 321–337. Birkhauser, Boston, MA, USA.

Brown, A. G. (1981). *Organisation in the Spinal Cord. The Anatomy and Physiology of Identified Neurones.* Springer-Verlag, Berlin.

Brown, G. L. & Eccles J. C. (1934*a*). The action of a single vagal volley on the rhythm of the heart beat. *Journal of Physiology* **81**, 211–240.

Brown G. L. & Eccles J. C. (1934*b*). Further experiments on vagal inhibition of the heart beat. *Journal of Physiology* **81**, 241–257.

Brown, G. L. & Guyenet, P. G. (1985). Electrophysiological study of cardiovascular neurones in the rostral ventrolateral medulla in rats. *Circulation Research* **56**, 359–369.

Cannon, W. B. (1932). *The Wisdom of the Body.* Norton, New York.

Champagnat, J., Denavit-Saubie, M., Grant, K. & Shen, K. F. (1986). Organization of synaptic transmission in the mammalian solitary complex, studied *in vitro*. *Journal of Physiology* **381**, 551–573.

Champagnat, J., Jacquin, T. & Richter, D. W. (1986). Voltage-dependent currents in neurones of the nuclei of the solitary tract of rat brainstem slices. *Pflügers Archiv* **406**, 372–379.

Cochrane, K. L. & Nathan, M. A. (1993). Cardiovascular effects of lesions of the rostral ventrolateral medulla and the nucleus reticularis parvocellularis in rats. *Journal of the Autonomic Nervous System* **43**, 69–82.

Coote, J. H. (1988). The organization of cardiovascular neurons in the spinal cord. *Review of Physiology, Biochemistry and Pharmacology* **110**, 147–285.

Coote, J. H., Hilton, S. M. & Zbrozyna, A. W. (1973). The ponto-medullary area integrating the defence reaction in the cat and its influence on muscle blood flow. *Journal of Physiology* **229**, 257–274.

Coote, J. H. & Macleod, V. H. (1974*a*). The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. *Journal of Physiology* **241**, 453–457.

COOTE, J. H. & MACLEOD, V. H. (1974b). Evidence for the involvement in the baroreceptor reflex of a descending inhibitory pathway. *Journal of Physiology* **241**, 477–496.

COX, G. E., JORDAN, D., MORUZZI, P., SCHWABER, J. S., SPYER, K. M. & TURNER, S. A. (1985). Amygdaloid influences on brain-stem neurones in the rabbit. *Journal of Physiology* **381**, 135–148.

CZACHURSKI, J., DEMBOWSKY, K., SELLER, H., NOBILING, R. & TAUGNER, R. (1988). Morphology of electrophysiologically identified baroreceptor afferents and second order neurones in the brainstem of the cat. *Archives Italiennes de Biologie* **126**, 129–144.

DALY, M. DE B. (1985). Interactions between respiration and circulation. In *Handbook of Physiology*, section II, *The Respiratory System*, pp. 529–594. American Physiological Society, Bethesda, MD, USA.

DAMPNEY, R. A. L. (1994). Functional organization of central pathways regulating the cardiovascular system. *Physiological Reviews* (in the Press)

DEUCHARS, J. & IZZO P. N. (1991). Demonstration of a monosynaptic pathway from the nucleus tractus solitarius to regions of the ventrolateral medulla with specific reference to vagal motoneurones in the nucleus ambiguus of the anaesthetized cat. *Journal of Physiology* **438**, 80*P*.

DEUCHARS, J., SPYER, K. M. & IZZO, P. N. (1992). Demonstration of substance P-containing axons in synaptic contact with vagal preganglionic neurones in the nucleus ambiguus of the anaesthetized rat. *Journal of Physiology* **452**, 223*P*.

DONOGHUE, S., FELDER, R. B., JORDAN, D. & SPYER, K. M. (1984). Processing of afferent inputs in cardiovascular control. *Clinical and Experimental Hypertension – Theory and Practice* **A6**, 173–184.

FELDBERG, W. S. (1982). *Fifty Years On: Looking Back on Some Developments in Neurohumoral Physiology*. Liverpool University Press, Liverpool.

FELDBERG, W. S. & GUERTZENSTEIN, P. G. (1972). A vasodepressor effect of pentabarbitone sodium. *Journal of Physiology* **224**, 83–103.

FELDBERG, W. S. & GUERTZENSTEIN, P. G. (1976). Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. *Journal of Physiology* **258**, 337–355.

GEBBER, G. L. (1990). Central determinants of Sympathetic nerve discharge. In *Central Regulation of Autonomic Functions*, ed. LOEWY A. D. & SPYER, K. M., pp. 126–144. Oxford University Press, New York.

GILBEY, M. P., JORDAN, D., RICHTER, D. W. & SPYER, K. M. (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *Journal of Physiology* **356**, 65–78.

GLYNN, I. M. (1993). All hands to the sodium pump. *Journal of Physiology* **462**, 1–30.

GRANATA, A. R. & KITAI, S. T. (1992). Intracellular analysis *in vivo* of different barosensitive bulbospinal neurons in the rat rostral ventrolateral medulla. *Journal of Neuroscience* **12**, 1–20.

GUERTZENSTEIN, P. G. & SILVER A. (1974). Fall in blood pressure produced from discrete regions of the central surface of the medulla by glycine and lesions. *Journal of Physiology* **242**, 489–503.

GUYENET, P. G. (1990). Role of the ventral medulla oblongata in blood pressure regulation. In *Central Regulation of Autonomic Functions*, ed. LOEWY, A. D. & SPYER, K. M., pp. 145–167. Oxford University Press, New York.

HESS, W. R. & BRUGGER, M. (1943). Das subkorticale zentrum der affektiven-abwehrreaktion. *Acta Physiologica Helvetica* **1**, 33–52.

HEYMANS, C. & NEIL, E. (1958). *Reflexogenic Areas of the Cardiovascular System*. Churchill, London.

HILTON, S. M. (1966). Hypothalamic regulation of the cardiovascular system. *British Medical Bulletin* **22**, 243–248.

HILTON, S. M. (1975). Ways of viewing the central nervous control of circulation – old and new. *Brain Research* **87**, 213–219.

HILTON, S. M. & JOELS, N. (1965). Facilitation of chemoreceptor reflexes during the defence reaction. *Journal of Physiology* **176**, 20–22*P*.

IZZO, P. N., DEUCHARS, J. & SPYER K. M. (1993). Localization of cardiac vagal preganglionic motoneurones in the rat: immunocytochemical evidence of synaptic inputs containing 5-hydroxytryptamine. *Journal of Comparative Neurology* **327**, 572–583.

IZZO, P. N., SYKES, R. M. & SPYER, K. M. (1992). γ-amino butyric acid immunoreactive structures in the nucleus tractus solitarius: A light and electron microscopic study. *Brain Research* **591**, 69–79.

JORDAN, D. (1990). Autonomic changes in affective behaviour. In *Central Regulation of Autonomic Functions*, ed. LOEWY A. D. & SPYER K. M., pp. 349–367. Oxford University Press, New York.

JORDAN, D., KHALID, M. E. M., SCHNEIDERMAN, N. & SPYER, K. M. (1982). The location and properties of preganglionic vagal cardiomotor neurones in the rabbit. *Pflügers Archiv* **395**, 244–250.

JORDAN, D. & SPYER, K. M. (1986). Brainstem integration of cardiovascular and pulmonary afferent activity. In *Progress in Brain Research*, vol. 67, *Visceral Sensation*, ed. CERVERO, F. & MORRISON J. F. B., pp. 295–314. Elsevier Science Publishers, Amsterdam.

JORDAN, D. J., MIFFLIN, S. W. & SPYER, K. M. (1988). Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. *Journal of Physiology* **399**, 389–404.

KALIA M. & RICHTER D. W. (1985a). Morphology of physiologically identified slowly adapting lung stretch afferents stained with intra-axonal horseradish peroxidase in the nucleus of the tractus solitarius of the cat. I. A light microscopic analysis. *Journal of Comparative Neurology* **193**, 467–508.

KALIA, M. & RICHTER, D. W. (1985b). Morphology of physiologically identified slowly adapting lung stretch afferents stained with intra-axonal horseradish peroxidase in the nucleus of the tractus solitarius of the cat. II. An ultrastructural analysis. *Journal of Comparative Neurology* **241**, 521–535.

LINDSEY, B. G., HERNANDEZ, Y. M., MORRIS, K. F. & SHANNON, R. (1992a). Functional connectivity between brain stem midline neurons with respiratory-modulated firing rates. *Journal of Neurophysiology* **67**, 890–903.

LINDSEY, B. G., HERNANDEZ, Y. M., MORRIS, K. F., SHANNON, R. & GERSTIN, G. L. (1992b). Respiratory-related neural assemblies in the brain stem midline. *Journal of Neurophysiology* **67**, 905–922.

LIPSKI, J., WALDVOGEL, H. J., PILOWSKI, P. & JIANG, C. (1990). GABA-immunoreactive boutons make synapses with inspiratory neurons of the dorsal respiratory group. *Brain Research* **529**, 309–314.

LOESCHCKE, H. H. (1982). Review Lecture: Central chemosensitivity and the reaction theory. *Journal of Physiology* **332**, 1–24.

LOEWY, A. D. (1990). Central autonomic pathways. In *Central Regulation of Autonomic Functions*, ed. LOEWY A. D. & SPYER, K. M., pp. 88–103. Oxford University Press, New York.

LOEWY, A. D. & SPYER, K. M. (1990). Vagal preganglionic neurons. In *Central Regulation of Autonomic Functions*, ed. LOEWY A. D. & SPYER, K. M., pp. 68–87. Oxford University Press, New York.

MCALLEN, R. M. (1985). Mediation of fastigial pressor response and a somatosympathetic reflex by ventral medullary neurones in the cat. *Journal of Physiology* **368**, 423–433.

MCALLEN, R. M. (1986a). Identification and properties of sub-retrofacial bulbospinal neurones: a descending cardiovascular pathway in the cat. *Journal of the Autonomic Nervous System* **17**, 151–164.

MCALLEN, R. M. (1986b). Location of neurones with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. *Neuroscience* **18**, 43–49.

MCALLEN, R. M. (1986c). Action and specificity of ventral medullary vasopressor neurones in the cat. *Neuroscience* **18**, 51–59.

McAllen, R. M. (1987). Central respiratory modulation of subretrofacial bulbospinal neurones in the cat. *Journal of Physiology* **388**, 533–545.

McAllen, R. M. & Dampney, R. A. L. (1989). The selectivity of descending vasomotor control by subretrofacial neurons. In *Progress in Brain Research*, vol. 81, *Central Neural Organization of Cardiovascular Control*, ed. Ciriello, J., Caverson, M. M. & Polosa, C., pp. 233–242. Elsevier Science Publishers, Amsterdam.

McAllen, R. M. & Dampney, R. A. L. (1990). Vasomotor neurons in the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed but not body region. *Neuroscience Letters* **110**, 91–96.

McAllen, R. M. & Spyer, K. M. (1976). The location of cardiac vagal preganglionic motoneurones in the medulla of the cat. *Journal of Physiology* **258**, 187–204.

McAllen, R. M. & Spyer, K. M. (1978*a*). Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *Journal of Physiology* **282**, 353–364.

McAllen, R. M. & Spyer, K. M. (1978*b*). The baroreceptor input to cardiac vagal motoneurones. *Journal of Physiology* **282**, 365–374.

McWilliam, P. M., Yang, T. & Chen, L. X. (1991). Changes in the baroreceptor reflex at the start of muscle contraction in the decerebrate cat. *Journal of Physiology* **436**, 549–558.

Maqbool, A., Batten, T. F. C., McWilliam, P. N. (1991). Ultrastructural relationships between GABAergic terminals and cardiac vagal preganglionic motoneurones and vagal afferents in the cat: a combined HRP tracing and immunogold labelling study. *European Journal of Neuroscience* **3**, 501–513.

Marks, S. A. & Gilbey, M. P. (1992). Effect on cardiac sympathetic nerve activity of phenylephrine microinjected into the cat intermediolateral cell column. *Journal of Physiology* **453**, 185–195.

Marks, S. A. & Morrison, S. F. (1993). Excitatory synaptic potentials evoked in sympathetic preganglionic neurones by stimulating the rostral ventrolateral medulla (RVLM) in the neonatal rat brainstem–spinal cord preparation. *Journal of Physiology* **473**, 155*P*.

Marshall, J. M. (1977). The cardiovascular response to stimulation of carotid chemoreceptors. *Journal of Physiology* **266**, 48–49.

Merrill, E. G., Lipski, J., Kubin L. & Fedorko, L. (1983). Origin of the expiratory inhibition of nucleus tractus solitarius inspiratory neurones. *Brain Research* **263**, 43–50.

Mifflin, S. W. (1992). The arterial chemoreceptor input to nucleus tractus solitarius. *American Journal of Physiology* **263**, R368–375.

Mifflin, S. W., Spyer, K. M. & Withington-Wray, D. J. (1988*a*). Baroreceptor inputs to the nucleus tractus solitarius in the cat: postsynaptic actions and the influence of respiration. *Journal of Physiology* **399**, 349–367.

Mifflin, S. W., Spyer, K. M. & Withington-Wray, D. J. (1988*b*). Baroreceptor inputs to the nucleus tractus solitarius in the cat; modulation by the hypothalamus. *Journal of Physiology* **399**, 369–387.

Morrison S. F. & Gebber, G. L. (1982). Classification of raphe neurons with cardiac-related activity. *American Journal of Physiology* **243**, R49–59.

Overton, E. (1902). Beiträge zur allgemeinen muskel- und nervenphysiologie. II. Ueber die unentbehrlichkeit von natrium- (oder lithium-) ionen für den contractionsact des muskels. *Pflügers Archiv* **92**, 346–386.

Paton, J. F. R., Silva-Carvalho, L., Goldsmith, G. E. & Spyer, K. M. (1990). Inhibition of barosensitive neurones evoked by lobule IXb of the posterior cerebellar cortex in the decerebrate rabbit. *Journal of Physiology* **427**, 553–565.

Paton, J. F. R. & Spyer, K. M. (1992). Cerebellar cortical regulation of circulation. *News in Physiological Sciences* **7**, 124–129.

Peiss, C. N. (1965). Concepts of cardiovascular regulation; past, present, future. In *Nervous Control of the Heart*, ed. Randall, W. C., pp. 154–197. Williams and Wilkins, Baltimore, MD, USA.

Richter D. W. & Spyer K. M. (1990). Cardiorespiratory control. In *Central Regulation of Autonomic Functions*, ed. Loewy, A. D. & Spyer, K. M., pp. 189–207. Oxford University Press, New York.

Richter, D. W., Spyer, K. M., Gilbey, M. P., Lawson, E. E., Bainton, C. R. & Wilhelm, Z. (1991). On the existence of a common cardiorespiratory network. In *Cardiorespiratory and Motor Co-ordination*, ed. Koepchen, H.-P. & Huopaniemi, T., pp. 118–139. Springer-Verlag, Berlin.

Ross, C. A., Ruggiero, D. A., Joh, T. H., Park, D. H. & Reis, D. J. (1983). Adrenaline synthesizing neurons in the rostral ventrolateral medulla: a possible role in tonic vasomotor control. *Brain Research* **273**, 257–262.

Ross, C. A., Ruggiero, D. A., Joh, T. H., Park, D. H. & Reis, D. J. (1984). Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *Journal of Comparative Neurology* **228**, 168–185.

Seller, H. & Illert, M. (1969). The localization of the first synapse in the carotid sinus baroreceptor reflex pathway and its alteration of the afferent input. *Pflügers Archiv* **306**, 1–19.

Silva-Carvalho, L., Dawid-Milner, M. S., Goldsmith, G. E. & Spyer, K. M. (1993). Hypothalamic-evoked effects in cat nucleus tractus solitarius facilitating chemoreceptor reflexes. *Experimental Physiology* **78**, 425–428.

Spyer, K. M. (1981*a*). Neural organisation and control of the baroreceptor reflex. *Reviews of Physiology, Biochemistry and Pharmacology* **88**, 23–124.

Spyer, K. M. (1981*b*). Central control of the cardiovascular system. In *Recent Advances in Physiology*, vol. 10, ed. Baker, P. F., pp. 163–200. Churchill Livingstone, Edinburgh.

Spyer, K. M. (1984). Central control of the cardiovascular system. In *Recent Advances in Physiology*, vol. 10, ed. Baker, P. F., pp. 163–200. Churchill Livingstone, Edinburgh.

Spyer, K. M. (1989). Neural mechanisms involved in cardiovasular control during affective behaviour. *Trends in Neurosciences* **12**, 506–513.

Spyer, K. M. (1990). The central nervous organization of reflex circulatory control. In *Central Regulation of Autonomic Functions*, ed. Loewy, A. D. & Spyer, K. M., pp. 168–188. Oxford University Press, New York.

Spyer, K. M. Izzo, P. M., Lin, R. J., Paton, J. F. R., Silva-Carvalho, L. F. & Richter, D. W. (1990). The central nervous organization of the carotid body chemoreceptor reflex. In *Chemoreceptors and Chemoreceptor Reflexes*, ed. Acker, H., Trzebski, A. & O'Regan, R. G., pp. 317–321. Plenum Press, New York .

Strack, A. M., Sawyer, W. B., Hughes, J. H., Platt, K. B. & Loewy, A. D. (1989*a*). A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Research* **491**, 156–162.

Strack, A. M., Sawyer, W. B., Platt, K. B. & Loewy, A. D. (1989*b*). CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labelling with pseudorabies virus. *Brain Research* **491**, 274–296.

Sun, M.-K. & Reis, D. J. (1993). Hypoxic excitation of medullary vasomotor neurons in rats are not mediated by glutamate or nitric oxide. *Neuroscience Letters* **157**, 219–222.

Sun M.-K. & Spyer, K. M. (1991). GABA-mediated inhibition of medullary vasomotor neurones by area postrema stimulation in rats. *Journal of Physiology* **436**, 669–684.

Sved, A. F., Tsukamoto, K. & Sved, J. C. (1993). $GABA_b$ receptors in the nucleus tractus solitarius in cardiovascular regulation. In *Central Neural Mechanisms in Cardiovascular Regulation*, vol. 2, ed. Kunos, G. & Ciriello, J., pp. 338–356. Birkhauser, Boston, MA, USA.

Timms, R. J. (1981). A study of the amygdaloid defence reaction showing the value of althesin anaesthesia in studies of the function of the fore-brain in casts. *Pflügers Archiv* **391**, 49–56.

ZAGON, A. & SMITH, A. D. (1993). Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. *Neuroscience* **54**, 729–743.

ZHOU, S.-Y., FUTURO-NETO, H. A. & GILBEY, M. P. (1992). Do caudal raphé neurones relay central respiratory–related inputs to sympathetic preganglionic neurones in the anaesthetized rat? *Journal of Physiology* **467**, 16*P*.

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